Immunohistochemistry Frozen Protocol

Preparation - Perfusion based tissue preparation

1. Fix tissue by perfusing the animal with freshly prepared 4% paraformaldehyde.

2. Cyroprotect the tissue by either directly perfusing a sucrose solution, or by first dissecting the tissue and allowing it to sink overnight in a 30% sucrose/70% fixative solution.

3. Embed tissue in OCT cryostat sectioning medium and store at -80° C until ready for sectioning. Tissue can be safely stored for 6-12 months.

4. When ready for sectioning, move the embedded tissue directly into the cryostat and use OCT medium to mount it to the chuck. Allow the temperature of the tissue to equilibrate with the cryostat.

5. Cut the tissue in 5-20 µm thick sections. Mount tissue sections onto gelatin or poly-L-lysine coated slides by placing the cold sections onto warm slides. Slides can be safely stored for 6-12 months at -80° C until ready for staining.

Preparation - Snap frozen based tissue preparation

1. After dissection, immediately snap freeze tissue with isopentane cooled by liquid nitrogen. To do this, prepare a small dewar of liquid nitrogen. Take an aluminum can cut in half and fill with isopentane. Float the can in the liquid nitrogen until the isopentane is cooled. Quickly dissect the tissue, wrap in aluminum foil, and place in the cooled isopentane. After the tissue is frozen, place it in dry ice and move to -80° C until ready for cutting.

2. Embed tissue in OCT compound by slowly layering the compound so that the tissue does not thaw. Move the embedded tissue directly into the cryostat and use OCT medium to mount it to the chuck. Allow the temperature of the tissue to equilibrate with the cryostat.

3. Cut the tissue in 5-20 µm thick sections. Mount tissue sections onto gelatin or poly-L-lysine coated slides by placing the cold sections onto warm slides. Slides can be safely stored for 6-12 months at -80° C until ready for fixing. Uncut tissue can be restored at -80° C.
4. Remove slides from freezer and fix with cold fixative (acetone or methanol) for 10 minutes. Proceed to staining.

Immunofluorescent staining

1. Warm slides to room temperature and wash slides twice with PBS.

2. Draw a circle on the slide around the tissue with a hydrophobic barrier pen or use rubber cement.

3. Wash the sections twice for 10 minutes with 1% animal serum in PBS-T (PBS with 0.4% Triton X-100). The species of the animal serum is dependent on the host of your secondary antibody. Ex. If using goat anti-mouse secondary, use goat serum.

4. Block any non-specific binding by incubating the tissue sections with 5% serum in PBS-T for 30 minutes at room temperature.

5. Add primary antibody diluted in 1% serum PBS-T and incubate overnight at 4° C. Use the recommended dilution of the antibody as specified on the datasheet. Do not let the tissue dry from this step forward or signal will be lost.

6. Wash sections twice with 1% serum PBS-T for 10 minutes each.

7. Dilute secondary antibody in 1% serum PBS-T and incubate with sections at room temperature for 1 hour. Use the recommended dilution of the antibody as specified on the datasheet.

8. Wash sections twice with 1% serum PBS-T for 10 minutes each.

9. Optional: Double/Nuclear labeling
    a. Double labeling: If using a second primary antibody, repeat steps 5-8.

    b. Nuclear labeling: After application of all primary antibodies, DNA binding dyes such as DAPI can be applied, which are used without the need for secondary antibodies. Use the recommended dilution and incubation time as specified on the datasheet. After incubation, wash once for 5 minutes with PBS.

10. Tap off excess wash and apply one drop of anti-fade mounting medium to the slide. Coverslip the tissue sections. Ring the edges of the coverslip with clear fingernail polish to prevent the cells from drying. Allow nail polish to air dry.

11. Slides may now be examined under a microscope with the appropriate fluorescent filter sets. Limiting the amount of time each slide is exposed to the microscope's light will aid in prolonging the signal and prevent photobleaching.

12. Slides can be stored between -20° C and 4° C in a dark slide box or slide book.